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(Original Article)

Physicochemical Properties of Oils Extracted from Two Moringa Cultivars Seeds

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Abstract

Moringa trees are found in many parts of the world, including tropical regions, and is a valuable plant for obtaining oil for food and non-food applications. This study aimed to investigate the physicochemical characteristics of oils extracted from moringa seeds. The results showed that *Moringa oleifera* oil had a refractive index of 1.4781 (at 25ºC), a density of 0.817 g/cm³, and a viscosity of 33.5 mPa/s. In contrast, *M. stenopetala* oil had a refractive index of 1.4678, a density of 0.820 g/cm³, and a viscosity of 32.0 mPa/s. The chemical characteristics of *M. oleifera* oil, such as acid value, peroxide value, iodine value, and saponification value, were 0.92, 1.71, 67.07, and 180.00, respectively. For *M. stenopetala* oil, these values were 0.99, 0.86, 54.40, and 209.00; respectively. Gas-liquid chromatography (GLC) was used to detect the fatty acids in the oils, revealing that oleic acid was predominant, followed by palmitic acid. Sterols such as beta-sitosterol and campesterol were also estimated in the moringa oils. Furthermore, the oils demonstrated good oxidative stability.

Keywords: *Moringa oleifera, Moringa stenopetala, Sterols, Oleic acid.*

Introduction

Moringa varieties are widely spread in Africa and South Asia. It is characterized by its ease of cultivation in most regions and climatic conditions, its high production, and the use of all parts of the plant. It is also used for social, nutritional, medicinal, and industrial purposes (Hamza and Azmach 2017; Saa *et al.* 2019).

Beyond the High content of carbohydrates, proteins and lipids, Moringa seeds are source of micronutrients, minerals and bioactive compounds such as sterols, flavonoids, phytates, and saponins. They also contain vitamins E, A and B_1 (Mbah *et al.* 2012; Saa *et al.* 2019).

Moringa seed oils have desirable properties for culinary purposes. Under the optimal conditions for extracting oil from moringa seeds and studying its

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Physicochemical properties, it was found to be suitable as edible oil for human consumption (Shumi and Bacha 2022).

The fatty acid composition of moringa seed oil falls into the categories of high-oleic oils which contain approximately 75% oleic acid, and it is characterized by its high content of unsaturated fatty acids compared to saturated fatty acids. The MUFA/SFA ratio is linked to a lower risk of cardiovascular death and stroke, and this ratio is distinctive for many oils (Leone *et al.* 2016).

The high content of oleic acid in moringa seed oils makes it stable when used for frying, as it produces low conjugated dienes and trienes compared to other vegetable oils containing a high percentage of unsaturated fatty acids. The oxidative stability of moringa seed oil depends on its low polyunsaturated fatty acids, high oleic acid, and tocopherol content. Oils from the oleic acid group have a good effect on coronary heart disease and are nutritionally desirable (Salama *et al.* 2020).

Moringa seed oils contain a high percentage of antioxidants with therapeutic properties such as tocopherols (α-, γ- and δ- tocopherols), with α-tocopherol predominance, the α-Tocopherol prevents lipid oxidation such as cellular components and polyunsaturated fatty acids in the body. Additionally, it prevents cardiovascular diseases (Wiltshire *et al.* 2022).

Moringa seed oil has a high antioxidant capacity and has potential for nutritional, industrial, and health applications. Generally, expanding the cultivation of moringa is an economic strategy to fight poverty (Gebino *et al.* 2021).

This study aimed at the extraction and characterization of moringa seed oils from two cultivars, namely *M. oleifera* and *M. stenopetala*. The cold pressing method used for oil extraction, then both chemical and physical characteristics of moringa seed oils have been estimated.

Materials and Methods

Moringa seeds

Seeds of two moringa cultivars, namely *Moringa oleifera* and *Moringa stenopetala,* were obtained from the farm of crops department, faculty of Agriculture, Minia University. Moringa seeds were cleaned manually by removing the foreign matter overlapping with seeds, the seeds were stored in tightly sealed bags in a cool, dry condition until use.

Chemicals and Reagents

All chemicals and reagents used (analytical grades) in this study were obtained from Sigma (St. Louis, USA), El Gomhouria Pharmaceutical Company and El-Nasr Pharmaceutical Chemicals Company, Assiut, Egypt.

Oil Extraction

A hydraulic press was used for cold pressing. The cylindrical press cage had 1 mm perforations and was 90 mm in diameter and 190 mm height. A

sedimentation process was carried out for a week to filter the extracted oil, as the composition and quality of the oil can be negatively affected by impurities. The oil was filtered through filter paper (Whatman No. 1), and the pure oil was stored in sealed brown bottles (Özcan *et al*. 2019).

Determination of physical properties

By an Abbe 60 refractometer at 25°C, the refractive Index was determined. Density and viscosity were determined by AOCS official methods AOCS (1997). Three samples were taken, and the color was determined by transmission measurement in a 1 in. cell using a Lovibond tintometer (Model-F, The Tintometer Ltd., Salisbury, U.K.) and calculated as 1× Yellow units + 5 ×Red units (Y+5R value). Average values were expressed as Lovibond units (LU). Color was estimated according to AOAC (2005).

Determination of chemical properties

The Peroxide value, acid value, iodine value and saponification number were determined in moringa seed oils according to AOCS (1998). TBA value was determined using UV-Visible spectrophotometry (C-7200, Peak Instruments, USA) at 532 nm using the method described by Guzman-Chozas *et al.* (1997). The ultraviolet absorbance at 232 nm (conjugated diene) and at 268 nm (conjugated triene) samples of oil were measured on spectronic-21 UV-Visible Spectrophotometer (C-7200, Peak Instruments, USA) as described by Danopoulos and Ninni (1972).

Determination of fatty acids composition

The methyl esters of fatty acids in moringa seed oils were separated using HP 6890 GC capillary column gas liquid chromatography (HP 6890 series GC) with a dual flame ionization were carried out on (60 m × 0.32 mm × 25 µm). Carrier gas was N_2 with flow rate 1.5 ml/min, splitting ratio of 1:50. The injector temperature was 250°C and that of Flame Ionization Detector (FID) was 280°C. The temperature setting was as follows: 150°C TO 210°C at 5°C/min, then held at 210°C for 25 min. Peaks were identified by comparing the retention times obtained with stander methyl esters (ISO 12966-2, 2017).

Calculation of the oxidative stability

The oxidative stability of the extracted oils based on unsaturated fatty acids (USFAs) content was calculated according to Fatemi and Hammond (1980) as follows:

Oxidizability = [oleate % + 10.3 (lioleate %) + 21.6 (liolenate %)] /100

Determination of vitamin E

Vitamin E (as alpha tocopherol) in moringa seed oils was determined using high performance liquid chromatography (HPLC) The HPLC system was equipped with an HP Agilent 1200 (Agilent Technologies, Palo Alto, CA, USA.), coupled with an Agilent 1100 Series fluorescence detector set at the wavelengths

λ = 295 and 330 nm for excitation and emission, respectively according to the ISO 9936 (1997) standard method.

Determination of sterol composition

Sterol separation in moringa seed oils was performed according to the Coi *et al.* (2017). The αcholestanol was used as an internal standard solution. Analyses were conducted through a Gas Chromatography (Agilent, HP 6890 series) equipped with a flame ionization detector (FID), using a DB-5 Agilent column (5% phenyl methyl polysiloxane, 30 m x 0.32 mm internal diameter × 0.25 µm film thickness). Helium was used as a carrier gas at a flow of 2 ml/min using the split-splitless injection. The injector/detector temperatures were held at 280 and 290 °C, respectively. The column temperature was set to 240 °C and it was next raised to 260 °C at a rate of 4 °C /min. Sterols peaks were identified according to COI (2017) and confirmed by GC–MS (NIST database, 2002) database whilst operating under similar conditions as to that of the GC-FID.

Estimation of total phenolics

In moringa seed oils, the total phenol content of the oil sample was determined using Folin Ciocalteu reagent, 1 mL aliquot of oil sample (100 µg mL) was put in test tube, 2.5 mL Folin-Ciocalteu-1 reagent (0.2 M) and 2 mL sodium carbonate (7.5%) was added, allowed to stand in the dark for 20 min at room temperature, thereafter the absorbance was read at 765 nm. The amount of total phenolic component in the oil sample was determined from gallic acid calibration curve and expressed as mg of Gallic acid Equivalent per gram sample (mg GAE g). by Khanahmadi *et al.* (2010).

Determination of total flavonoids

The total flavonoids content of the methanolic extract from moringa seed oils was determined using aluminum trichloride colorimetric method using rutin as standard (Nile and Khobragade, 2010). The method was based on the formation of a flavonoid-aluminum complex. The sample (0.1 mL) in methanol (100 µg/mL) was mixed with 0.2-1 mL of 5% sodium nitrate, then allowed to react for 5 min thereafter 0.2 mL aluminum trichloride in methanol (10%) and 1 mL of sodium hydroxide (1 M) were added, then allowed to stand at room temperature for 15 min. The absorbance was read at 510 nm against reagent blank. The amount of flavonoid was calculated from rutin calibration curve, results expressed as mg of Rutin Equivalent per gram of sample (mg RE/g).

Determination of antioxidant activity by DPPH

In sample moringa seed oils, the radical scavenging activities of the extracts against 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical were determined by Güllüce *et al*. (2004**)** at 517 nm. According to the formula:

%DPPH scavenging = [(AC – AS) / AC] × 100

Where AC is the absorbance of the control reaction and AS is the absorbance when the sample extract is added.

Statistical analysis

Statistical analysis: Analyses were carried out in triplicates of each set up. Results are expressed as Mean ±SEM. Statistical significant difference ($p<0.05$) was determined using student's t-test. All data were analyzed using Statistical Package for the Social Science 15.0 for windows (SPSS 15.0) Mishra *et al*. (2019).

Results and Discussion

Physical properties of moringa seed oils

The whole moringa seeds of both cultivars showed an oil yield of 25.00% (*M. oleifera*) and 29.00% (*M. stenopetala*). In Table 1, it is shown that there is a highly significant difference ($P < 0.05$) between the *M. oleifera* and *M. Stenopetala* cultivars in both viscosity and red and yellow color units. On the contrary, there was no significant ($P > 0.05$) between the two cultivars in refractive index and density, as it was shown that the examined color of oil showed red and yellow Lovibond units (1.7, 27.8) for *M. oleifera* and (1.4, 18.8) for *M. stenopetala*; respectively. Refractive index (25ºC), density (g/cm^3), and viscosity (mPa/s) were (1.4781, 0.817 and 33.5) for *M. oleifera* oil and (1.4678, 0.820 and 32.0) for *M. stenopetala* oil; respectively. During the cold press extraction, a higher viscosity is due to the water bound in the oil during extraction, while the higher acidity is attributed to the water added during the milling of the seeds prior to cold pressing. Indeed, the water addition enhances the lipolytic enzyme action and prolongs the contact of the seed (milled before cold pressing) with air and temperature. Nevertheless, the acidity of the cold-pressed oil is generally moderate, indicative of its good resistance to hydrolysis (Boukandoul *et al.* 2018). These results were confirmed by Lalas *et al.* (2003) and Rahman *et al.* (2009).

Table 1. Physical properties of moringa seed oils.

Characteristics	Samples	
	M. oleifera	***M. stenopetala***
Refractive index (25ºC)	1.4781^a±0.058	1.4678^a±0.058
Density (g/cm^3; 25ºC)	0.817^a±0.006	0.82^a±0.006
Viscosity (mPa/s; 25ºC)	33.5^a±0.289	32^b±0.115
Color red (LU)	1.7^a±0.058	1.4^b±0.029
Color yellow (LU)	27.8^a±1.039	18.8^b±0.404

$^{a\text{-}b}$Means ±SD (standard deviation) with different small letters in the same row differ significantly at $p<0.05$.

Chemical properties of moringa seed oils

The acid value indicates the presence of free fatty acids (FFA) in oil. High FFA content reduces the smoke point of the oil and makes it susceptible to oxidation. As shown in Table 2 there is a highly significant difference ($P < 0.05$) between the *M. oleifera* and *M. Stenopetala* cultivars in saponification value (mg KOH/g), iodine value (gI_2/100g), peroxide value (meq O_2/Kg) and acid value (mg oleic acid/g) for *M. oleifera* were 180.00, 67.07, 1.7, and 0.92. For *M. stenopetala* the corresponding values were 209.00, 54.40, 0.86, and 0.99; respectively. These results are in accordance with Boukandoul *et al.* (2017)

The thiobarbituric acid (TBA) test is used to detect secondary oxidants that cause off-flavour. The response of malonaldehyde with TBA indicates the level of aldehyde in the oil. Hydroperoxides in polyunsaturated fatty acids are formed by double bonds, which are measured by absorption using a UV spectrum. Measurement of conjugated dienes and trienes is a good parameter for evaluating the stability of fats and oils (Mohdaly *et al.* 2010).

The TBA (thiobarbituric acid), conjugated diene, and conjugated triene contents were present in quantities close to each other in both cultivars under study (Table 2). The *M. oleifera* was significantly ($P < 0.05$) higher in TBA (mg malonaldehyde/kg oil) and diene with values 0.50, 1.691 while *M. stenopetala* was significantly ($P < 0.05$) higher in triene with value 1.554. These results are in accordance with Manzoor *et al.* (2007) and Banerji *et al.* (2009).

Table 2. Chemical properties of moringa seed oils

Parameter	Samples	
	M. oleifera	***M. stenopetala***
Acid value (mg oleic acid/g)	$0.92^{b}\pm0.006$	$0.99^{a}\pm0.001$
Peroxide value (meq O_2/Kg)	$1.71^{a}\pm0.012$	$0.86^{b}\pm0.012$
Saponification number (mg KOH/g)	$180^{b}\pm0.577$	$209^{a}\pm1.155$
Iodine value (gI_2/100g)	$67.07^{a}\pm1.744$	$54.4^{b}\pm1.212$
TBA (mg malonaldhyde/kg oil)	$0.5^{a}\pm0.029$	$0.25^{b}\pm0.023$
Conjugated diene	$1.691^{a}\pm0.001$	$1.618^{b}\pm0.01$
Conjugated triene	$1.238^{b}\pm0.002$	$1.554^{a}\pm0.013$

[a-b]Means ±SD (standard deviation) with different small letters in the same row differ significantly at $p<0.05$.

Fatty acids composition in moringa seed oils

As indicated in the obtained data Table 3, the total saturated fatty acids content was 20.76% and 23.13%. Palmitic acid is dominating with values of 7.28% and 10.93% for *M. oleifera* and *M. stenopetala* oils; respectively. Behenic, stearic and arachidic acids were recorded (5.04% and 3.88%), (4.57% and 4.37%) and (2.80% and 2.70%) in *M. oleifera* and *M. stenopetala* oils; respectively.

The percentage of polyunsaturated fatty acids was low, such as linoleic and linolenic acids with values (0.67% and 0.60%) and (0.15% and 0.07%) for *M. oleifera* and *M. stenopetala* oils; respectively. It is known that moringa seed oil belongs to the group of oleic acid oils, and accordingly, as shown in Table 3, the percentage of oleic acid in *M. oleifera* was 74.45% and in *M. stenopetala* 72.00%. These results are consistent with Gharsallah *et al.* (2021).

Table 3. Fatty acid composition of moringa seed oils (%)

Fatty acid (F.A)	Carbon: chain	Samples	
		M. oleifera	*M. stenopetala*
Palmitic	16:0	7.28	10.93
Stearic	18:0	4.57	4.37
Arachidic	20:0	2.80	2.70
Behenic	22:0	5.04	3.88
Lignoceric	24:0	1.07	1.25
Total saturated		20.76	23.13
Plmitoleic	16:1	1.94	2.16
Oleic	18:1	74.45	72.00
Linoleic	18:2	0.67	0.60
Linolenic	18:3	0.15	0.07
Gadoleic	20:1	1.90	1.98
Total unsaturated		79.11	76.81
T. Uns/T.s.		3.81	3.32
Oxidizability		0.85	0.80

Oxidative Stability of Oils

Oxidative stability is important for evaluating the qualities of oils and fats. The induction period expresses the purity and oxidative stability of edible oils and fats, induction period measured by the Rancimat method, through which oxidative stability standards were measured (Anwar *et al.* 2003).

As indicated in Table 4, the induction periods of the tested oils were 49.47 h and 75.58 h for *M. oleifera* and *M. stenopetala*, respectively. Therefore, the tested moringa oils exhibited good resistance to the oxidative rancidity. These results are in agreement with those obtained by Boukandoul *et al.* (2017).

Table 4. Induction time of moringa seed oils using the Rancimat apparatus

Oil sample	Temperature	Induction time (h)
M. oleifera	110ºC	49.47
M. stenopetala	110ºC	75.58

Sterols and vitamin E contents in moringa seed oils

Plant sterols or phytosterols (PS) are present in all plants and in food products of plant origin. Phytosterols regulate the fluidity and permeability of membranes and play an important role in adaptation of membranes to temperature. (Dutta *et al*. 2006). The sterols profile and vitamin E of studied oils is shown in Table 5. The sterol fraction of moringa seed oils mainly consisted of β-sitosterol, stigmasterol, campesterol and heptacosane for *M. oleifera* were 37.19%, 21.35%, 13.84%, and 6.35% and for *M. stenopetala* were 60.36%, 12.13%, 11.71%, and 3.20%; respectively. The results show that there is a highly significant difference ($P<0.05$) in both Beta-Sitosterol, Stigmasterol, and Heptacosane only.

Vitamins are a scavenger of lipid peroxidation radicals by preventing the production of hydroperoxides from singlet oxygen or destroying peroxyl radicals. In plants, the main source of vitamin E is α-tocopherol. It is found in cellular membranes and in lipoproteins. (Elmadfa and Wagner 2003).

The HPLC analysis showed that high significant difference ($P < 0.05$) between *M. oleifera* seed oils and *M. stenopetala* of vitamin E. This means that *M. oleifera* seed oils contain a good content of tocopherols and sterols, which supports the oxidative stability of the oil. Lalas and Tsaknis (2002) agreed with our results.

Table 5. Sterol and tocopherol contents in moringa seed oils (%)

Components	Samples	
	M. oleifera	***M. stenopetala***
Beta-Sitosterol	37.19^{b}±2.309	60.36^{a}±1.732
Stigmasterol	21.35^{a}±0.577	12.13^{b}±0.577
Campesterol	13.84^{a}±0.485	11.71^{a}±0.491
Heptacosane	6.35^{a}±0.202	3.20^{b}±0.346
Vitamin E(α-Tocopherol)	10.90^{a}±0.520	2.19^{b}±0.294

$^{a-b}$Means ±SD (standard deviation) with different small letters in the same row differ significantly at $p<0.05$.

Phenolic content and antioxidant activity of moringa seed oils

Table 6 indicates that *M. oleifera* oil phenolic and flavonoids content is highly significant difference ($P < 0.05$) higher than those of *M. stenopetala*. Total phenolic (mg GAE/100g oil) and total flavonoids (mg querecetin/100g oil) were 996.94 and 42.60 for *M. oleifera* and for *M. stenopetala* were 482.20 and 38.50; respectively. As for the antioxidant activity by the DPPH, it also was higher in the *M. oleifera* than the *M. stenopetala* due to its high contents of antioxidant parameters which led to an increase in activity compared to the *stenopetala* oil. The results regarding the stability of the oil against oxidation agreed with Latif *et al*. (2011) and Ahmed *et al*. (2016).

Table 6. Oxidative stability parameters of moringa seed oils

Analysis	Samples	
	M. oleifera	***M. stenopetala***
Total phenolics (mg GAE /100g oil)	996.94^{a}±46.188	482.20^{b}±34.641
Total flavonoids (mg quercetin /100g oil)	42.60^{a}±0.751	38.50^{b}±0.289
DPPH (%)	56.25^{a}±0.577	53.12^{b}±0.577

$^{a-b}$Means ±SD (standard deviation) with different small letters in the same row differ significantly at $p<0.05$.

Conclusion

The data obtained in this study useful for determining the physical and chemical properties of the oils extracted from two types of Moringa seeds, as it was found that *Moringa oleifera* cultivar surpassed *Moringa stenopetala* cultivar in most characteristics, particularly density, acid value, and peroxide value. In general, the moringa oils under study exhibited a good resistance to the oxidative rancidity.

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الخصائص الفيزيوكيميائية للزيوت المستخلصة من بذور صنفين من المورينجا

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الملخص

توجد شـــجرة المورينجا في العديد من أنحاء العالم، بما في ذلك المناطق الاســـتوائية، وهي نبات واعد للحصول على الزيت للتطبيقات الغذائية وغير الغذائية. هدفت هذه الدراسة إلى التحقيق في الخصـائص الفيزيائية والكيميائية للزيوت المسـتخرجة من بذور المورينجا. أظهرت النتائج أن زيت *M. oliefera* له معامل انكسـار 1.4781 (عند 25 درجة مئوية)، وكثافة 0.817 جم / سـم^3، ولزوجة 33.5 مللي باســـكال / ثانية. في المقابل، كان لزيت *M. stenopetala* معامل انكســـار 1.4678، وكثافة 0.820 جم / سم^3، ولزوجة 32.0 مللي باســـكال / ثانية. كانت الخصـــائص الكيميائية لزيت *M. oleifera* مثل الحموضـة، قيمة البيروكسـيد، قيمة اليود وقيمة التصـبن 0.92، 1.71، 67.07، 180.00; على التوالي. بالنســـبة لزيت *M. stenopetala* كانت هذه القيم 0.99، 0.86، 54.40، 209.00 على التوالي. تم اسـتخدام كروماتوغرافيا الغاز السـائل (GLC) للكشـف عن الأحماض الدهنية في الزيوت، وكشـفت أن حمض الأوليك كان سـائدًا، يليه حمض البالمتيك. كما تم تقدير السـتيرولات مثل بيتا سـيتوسـتيرول وكامبسـتيرول في زيوت المورينجا. علاوة على ذلك، أظهرت الزيوت استقرارًا أكسديًا جيدًا.

الكلمات المفتاحية: *المورينجا اوليفيرا، المورينجا ستينوبيتالا، الستيرولات، حمض الاوليك*